

AMENDMENTS TO THE CLAIMS

This listing of claims replaces all prior versions, and listings, of claims in the application.

1. (Currently Amended) A method of refolding an insoluble, recombinant, eukaryotic $\alpha(2,3)$ sialyltransferase (ST3Gal3) protein, wherein the ST3Gal3 protein comprises a maltose binding protein domain (MBD), the method comprising the steps of

(a) solubilizing the insoluble, recombinant, eukaryotic ST3Gal3 protein in a solubilization buffer; and

(b) contacting the soluble eukaryotic ST3Gal3 protein with a refolding buffer comprising a redox couple and poly(ethylene glycol) (PEG) and/or lauryl maltoside to refold the eukaryotic ST3Gal3 protein,

wherein the refolded eukaryotic ST3Gal3 protein catalyzes the transfer of a sialic acid sugar from a donor substrate to an acceptor substrate.

2.-4. (Canceled).

5. (Currently Amended) The method of claim 1, wherein the ~~first~~ eukaryotic ST3Gal3 protein further comprises a purification domain selected from the group consisting of a starch binding domain, a thioredoxin domain, a SUMO domain, a poly-His domain, a myc epitope domain, and a glutathione-S-transferase domain.

6. (Canceled).

7. (Previously Presented) The method of claim 1, wherein the eukaryotic ST3Gal3 protein is expressed in a bacterial host cell as an insoluble inclusion body.

8. (Previously Presented) The method of claim 1, wherein a second insoluble, recombinant eukaryotic glycosyltransferase is refolded with the eukaryotic ST3Gal3 protein.

9. (Previously Presented) The method of claim 8, wherein a third insoluble, recombinant eukaryotic glycosyltransferase is refolded with the eukaryotic ST3Gal3 protein and the second eukaryotic glycosyltransferase.

10. (Original) The method of claim 1, wherein the redox couple is selected from the group consisting of reduced glutathione/oxidized glutathione (GSH/GSSG) and cysteine/cystamine.

11. (Previously Presented) The method of claim 1, wherein the acceptor substrate is selected from the group consisting of a protein, a peptide, a glycoprotein, and a glycopeptide.

12.-13. (Canceled).

14. (Currently amended) The method of claim 1, wherein the donor substrate is a CMP-sialic acid PEG molecule and the acceptor substrate is selected from the group consisting of a protein, a peptide, a glycoprotein, and a glycopeptide.

15.-33. (Canceled).

34. (New) A method of refolding an insoluble, recombinant, eukaryotic $\alpha(2,3)$ sialyltransferase (ST3Gal3) protein, wherein the ST3Gal3 protein comprises a maltose binding protein domain (MBD) and is truncated to remove all or a portion of a stem region, the method comprising the steps of

(a) solubilizing the insoluble, recombinant, eukaryotic ST3Gal3 protein in a solubilization buffer; and

(b) contacting the soluble eukaryotic ST3Gal3 protein with a refolding buffer comprising a redox couple and poly(ethylene glycol) (PEG) and/or lauryl maltoside to refold the eukaryotic ST3Gal3 protein,

wherein the refolded eukaryotic ST3Gal3 protein catalyzes the transfer of a sialic acid sugar from a donor substrate to an acceptor substrate.

35. (New) A method of refolding an insoluble, recombinant, eukaryotic $\alpha(2,3)$ sialyltransferase (ST3Gal3) protein, wherein the ST3Gal3 protein comprises a maltose binding protein domain (MBD) and wherein an unpaired cysteine is removed by substitution with a non-cysteine amino acid, the method comprising the steps of

(a) solubilizing the insoluble, recombinant, eukaryotic ST3Gal3 protein in a solubilization buffer; and

(b) contacting the soluble eukaryotic ST3Gal3 protein with a refolding buffer comprising a redox couple and poly(ethylene glycol) (PEG) and/or lauryl maltoside to refold the eukaryotic ST3Gal3 protein,

wherein the refolded eukaryotic ST3Gal3 protein catalyzes the transfer of a sialic acid sugar from a donor substrate to an acceptor substrate.

36. (New) The method of claim 1, wherein the refolding buffer comprises PEG and lauryl maltoside.

37. (New) The method of claim 36, wherein the refolding buffer comprises about 0.02-10 mM reduced glutathione (GSH), 0.005-10 mM oxidized glutathione (GSSG), 0.005-10 mM lauryl maltoside, 50-250 mM NaCl, 2-10 mM KCl, 0.01-0.05% PEG 3350, and 150-550 mM L-arginine.

38. (New) The method of claim 34, wherein the refolding buffer comprises PEG and lauryl maltoside.

39. (New) The method of claim 38, wherein the refolding buffer comprises about 0.02-10 mM reduced glutathione (GSH), 0.005-10 mM oxidized glutathione (GSSG), 0.005-10 mM lauryl maltoside, 50-250 mM NaCl, 2-10 mM KCl, 0.01-0.05% PEG 3350, and 150-550 mM L-arginine.

40. (New) The method of claim 35, wherein the refolding buffer comprises PEG and lauryl maltoside.

41. (New) The method of claim 40, wherein the refolding buffer comprises about 0.02-10 mM reduced glutathione (GSH), 0.005-10 mM oxidized glutathione (GSSG), 0.005-10 mM lauryl maltoside, 50-250 mM NaCl, 2-10 mM KCl, 0.01-0.05% PEG 3350, and 150-550 mM L-arginine.